

# INVESTIGATOR'S ANNUAL REPORT

## National Park Service

All or some of the information provided may be available to the public

<b>Reporting Year:</b> 2005	<b>Park:</b> Shenandoah NP
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<b>Permit#:</b> SHEN-2004-SCI-0008	
<b>Park-assigned Study Id. #:</b> SHEN-00295	
<b>Project Title:</b> Floral Scents of Hybrids: Bridge or Barrier to Interspecific Gene Flow?	
<b>Permit Start Date:</b> Jun 02, 2004	<b>Permit Expiration Date</b> Sep 01, 2008
<b>Study Start Date:</b> Jun 02, 2004	<b>Study End Date</b> Sep 01, 2008
<b>Study Status:</b> Continuing	
<b>Activity Type:</b> Research	
<b>Subject/Discipline:</b> Ecology (Aquatic, Marine, Terrestrial)	
<b>Objectives:</b> <p>Recent advances in the application of transgenic crops have heightened awareness of the potential ecological impacts of these emerging technologies. One area of concern is the risk of unintended gene flow from genetically modified crops to unintended target plants or wild relatives. Basic research in the evolutionary processes of speciation and hybridization may have important insights to offer investigators of this phenomenon. The behavior of insect pollinators is among the most significant factors that determine whether two populations or species are reproductively isolated. There is a rich history of research on what factors attract insects to flowers. However, only recently, with the use of powerful genetic and statistical tools, have ecologists and evolutionary biologists been able to accurately track gene flow in populations and accurately measure the contribution of individual plant traits to reproductive success. Integrative research on plant morphology, genetics, and pollinator interactions has now shown how a few plant traits, such as the color and shape of flowers, affect reproductive barriers and gene flow. However, one trait that is extremely important as a pollinator attractant, floral scent, has gone largely unstudied from this perspective.</p> <p>We propose to use species of <i>Asclepias</i> (milkweeds) to investigate how floral scent is inherited by hybrids and how scents affect gene flow between species. Do the scents of hybrids accelerate or impede the rate of gene flow between species? A wealth of prior research has documented the effects of floral traits on the reproductive success of milkweeds and the roles of pollinators in mediating these effects. Milkweeds provide an illuminating system because of the rarity of successful hybridization, even though many species co-occur over vast areas. Even in the system in which we are working, in which hybridization has been well documented, there are strong barriers to hybridization. It has been hypothesized that rare F1 hybrids have morphological characteristics that bridge reproductive isolation.</p> <p>We propose to rigorously test the roles of hybrids as bridges promoting gene flow between species, with emphasis on an important, but often overlooked, attribute of floral morphology--floral scent. Our experiments will provide novel insights into the phenotypic characteristics of hybrids, the underlying genetics of these traits, the effects of these traits on the patterns of mating among hybrids and their parental populations, and the impact of these mating patterns on gene flow between species. Using a combination of observational studies of natural populations, controlled crossing experiments, and controlled pollination experiments, we will integrate mechanistic and realistic explanations for the affect of hybrid scents on gene</p>	

flow.

The proposed research will have significant impacts on training, outreach to underrepresented groups, and applied scientific disciplines. Mississippi State University provides an excellent opportunity to increase the research opportunities of African American students. The postdoctoral associate and undergraduate students will attend national meetings to present the results of their contributions to the project. The results of the proposed research will likely impact disciplines beyond ecology and evolutionary biology. Insights from this investigation could make a significant contribution to safely cultivating genetically engineered crops. Also, there are several threatened and endangered species of *Asclepias*, and this genus is known as a crucial food plant of the Monarch butterfly, which is of considerable conservation concern. A better understanding of the reproductive biology and hybridization dynamics of *A. exaltata* and *A. syriaca* may contribute to the conservation of rare milkweeds and the insects that depend on them.

#### Findings and Status:

In 2005 we augmented data collected in 2004 and conducted more detailed analyses. We collected data on the identities of insect pollinators to *Asclepias exaltata*, *A. syriaca*, and their hybrid, the rates of flower visitation by each pollinating species, and the rates of pollen transfer effected by each pollinating species. Pollination rates were measured for both parental plant species and their hybrid. Floral scent samples were collected from both parental species and their hybrids and these samples were analyzed by gas chromatography-mass spectroscopy. DNA samples were collected from 150 individuals of both parental species and hybrids. Sixty-nine plants were observed for 30 min observation periods during which pollinator species were identified and the duration of their foraging visits to flowers were measured. Insect visitation rates did not differ among plant species and their hybrids. Insect species differed significantly in the rates at which they visited flowers, regardless of plant species. Bees (honeybees and bumblebees) had greater visitation rates on *A. exaltata* than *A. syriaca* and hybrids. *A. syriaca* had higher visitation rates of a butterfly (silver-spotted skipper) and small bees. Hybrids had higher visitation rates of leaf-cutter bees. Plants were also observed to measure the rates of pollen removal and deposition per insect visit. The parental species and hybrids did not differ in rates of pollen removal. However, rates for different insect visitors did differ, depending on the identity of the plant. Honeybees had relatively low removal rates on *A. exaltata*, bumblebees had low removal rates on hybrids, and silver-spotted skippers had relatively high removal rates on *A. exaltata*. Overall, these results indicate strong year-to-year variation in the composition of the pollinating fauna.

Over 50 floral scent samples were collected from both species and their hybrid, including replicates collected at different periods of the day or night or using different methods. Scents were collected from flowers in the field by bagging flowers and creating a continuous airflow over the flowers and through an adsorbent filter using a pump. Collected scents were eluted into a solvent and transported to University of South Carolina for analysis in the lab of Dr. Rob Raguso. Samples collected in 2005 verified results from 2004 and provided fine scale resolution of differences in scent chemistry. We verified that both species and their hybrid differed in floral scent composition. *Asclepias syriaca* has a scent composed of a large number of compounds emitted at a high rate. *Asclepias exaltata* has few compounds, emitted at a low rate. Hybrid plants have strong scents reminiscent of *A. syriaca*. Our new analyses detected several compounds found only at trace levels in *A. syriaca* are present at very high levels in the hybrid. We found no difference between daytime and nighttime collections of scent from the same individuals. We also documented variation in scent production among organs within flowers of a species and different patterns of variation among parental species and hybrids.

Using species-specific chloroplast DNA sequences, we determined that hybrid individuals may be produced with either *A. exaltata* or *A. syriaca* as the seed parent, but that local populations may exhibit strong biases with respect to the ratio of hybrids mothered by the two parental species. This indicates that many hybrid populations may be quite young and/or the result of a single hybridization event. We have yet to find evidence of frequent introgression of chloroplast DNA from one species into another. We have developed microsatellite loci to further investigate gene flow between the parental species, but have not yet scored many plants. These markers will provide significantly more resolution of mating patterns and gene flow than has been possible with the chloroplast sequence data.

#### For this study, were one or more specimens collected and removed from the park but not destroyed during analyses?

Yes

#### Funding provided this reporting year by NPS:

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#### Funding provided this reporting year by other sources:

42000

#### Fill out the following ONLY IF the National Park Service supported this project in this reporting year by providing money to a university or college

#### Full name of college or university:

n/a

#### Annual funding provided by NPS to university or college this reporting year:

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